REMARKS

Claims 86-104 and 106-118 remain pending. Favorable reconsideration is respectfully requested.

The objections to Claim 106 and Claims 116-118 are believed to be obviated by the amendment submitted above.

Claim 106 has been amended to delete the reference to strain I-2025.

Claim 116 has been amended to recite "one of the unconventional amino acids present in the culture medium of step (b) is represented by an amino acid of formula I having L configuration."

Step a) of Claim 108 is a selection process according to Claim 86 by which cells are selected for their ability to incorporate an unconventional amino acid, which restores the functionality of a protein essential to growth. The unconventional amino corresponds to the amino acid which is initially encoded by the target condon. The resulting cells are mutated in the protein translation machinery (ex: ARNt-synthetase genes, see also page 7, paragraph 1), more particularly in their editing domain.

In step b), the cells selected in step a) are further cultivated with unconventional amino acids, in order to produce proteins comprising one of these unconventional amino acids.

In the sense of the present invention, an unconventional amino acid is an amino acid other than the amino acid, which should be normally incorporated at a given site with regard to the translated nucleic acid sequence. See page 2, lines 31 to 39 of the present specification.

It is not mentioned that the unconventional amino acids used in steps a) and b) are necessarily the same. On the contrary, it is recited on page 10, lines 11 to 21 that the unconventional amino acids may have specific additional properties. In example 6, for

instance, the mutated cells missincorporating Valine in place of Cystein (obtained according to the process of Claim 86 - Example 3) are cultivated in a culture medium containing the non canonical amino acids L-aminobutyrate.

In fact, the unconventional amino acids used in Claims 116 to 118 are those referred to in step b) of Claim 108, and not the one encoded by the target condon in step a).

In order to make this point clear, it has been specified in Claims 108 and 116 that the unconventional amino, acid carried out in Claims 108 and 116, is actually the one present in the culture medium of step b).

In view of the foregoing, withdrawal of the objections is respectfully requested.

The rejection of Claims 86-118 under 35 U.S.C. §112, first paragraph, is believed to be obviated by the amendment submitted above. Claim 86 has been amended to specify a bacterial or yeast cell, which the Examiner has indicated as allowable (see page 3 of the Official Action dated January 31, 2006). Accordingly, withdrawal of this ground of rejection is respectfully requested.

The rejection of Claims 103-105 and 107 under 35 U.S.C. §112, first paragraph, is believed to be obviated by the amendment submitted above.

Concerning the matter of the reproducibility of the invention to any aminoacyl-tRNA synthetase, aminoacyl-tRNA synthetase genes are well known in the art, especially among bacterial and yeast species. Thus, it would not be an undue burden for one skilled in the art to adapt the teaching provided in the specification concerning valyl-tRNA synthetase (ValS) to other aminoacyl-tRNA synthetase genes.

Furthermore, as reported in the enclosed review of Aminoacyl-tRNA synthetase (Delarue, M., 1995, *Current Opinion in Structural Biology*, 5:48-55), it is also well known that, despite the different topologies found in Aminoacyl-tRNA synthetase, their active sites (editing domain) and mechanisms of action are similar (see in particular the abstract).

In view of the foregoing, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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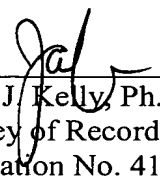
Norman F. Oblon



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Aminoacyl-tRNA synthetases

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Detailed mechanisms for each step of the reaction catalyzed by both class I and class II aminoacyl-tRNA synthetases have been proposed on the basis of crystallographic data of aminoacyl-tRNA synthetases in complex with their different substrates. Despite the very different topologies of the two classes, there are striking and unanticipated chemical similarities between their active sites and proposed mechanisms.

Current Opinion in Structural Biology 1995, 5:48–55

Introduction

The aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes that ensure the proper attachment of an amino acid to its cognate tRNA, generating the pool of charged tRNAs that are essential for mRNA translation into proteins. AaRSs catalyse the charging of tRNAs in a two-step reaction. First, ATP reacts with the amino acid to form an aminoacyl-adenylate (and diphosphate). Second, the activated amino acid is transferred to the tRNA to form the aminoacyl-tRNA and AMP. Until 1989, only three structures of aaRSs were known (^{bs}TyrRS [1,2], ^{ec}MetRS [3], and the ^{ec}GlnRS–tRNA^{Gln} complex [4], where bs and ec in superscript stand for *Bacillus subtilis* and *Escherichia coli*, respectively). They all share a structural domain called the Rossmann fold, originally observed in dehydrogenases, which we now know to be a characteristic of the so-called class I aaRS family, which contains 10 members [5•]. A major breakthrough in our understanding of aaRSs came from the structural determination of SerRS [6] and AspRS [7]; both of these enzymes lack the Rossmann fold and represent a second type of aaRSs (class II). Simultaneously, sequence analysis showed that all known aaRSs can be assigned to one or the other of these two classes ([8]; Table 1).

Much progress has been made in the past year on structural and functional studies of aaRSs. The new structures of class I aaRSs include GlnRS–tRNA^{Gln} in complex with ATP and Mg^{2+} [9••] and TrpRS in complex with tryptophanyl-adenylate [10••]. New class II structures include: *Thermus thermophilus* SerRS (^{tt}SerRS) alone [11•], as a complex with two analogues of the seryl-adenylate [12••] or with tRNA^{Ser} [13••]; ^{sc}AspRS–tRNA^{Asp} bound to ATP and Mg^{2+} or ^{sc}AspRS (where the sc in superscript stands for *Saccharomyces cerevisiae*) in complex with an amino acylated tRNA [14••]; ^{tt}AspRS alone [15•] or with aspartyl-adenylate [16••]. ^{tt}PheRS [17••], although at a preliminary stage of refinement, provides another example of a class II aaRS structure (Table 1).

In this review, I will first focus on the structural details that have been recently observed in different adenylate–aaRS complexes (one in each class); it is likely that the enzymatic reaction mechanisms that have been proposed [9••,14••] in these systems will hold for all aaRSs. I will also emphasize the extraordinary similarity of the active site of different enzymes and some aspects of the amino acid binding pockets. The use of these data to redesign amino acid specificity will be discussed.

In the second part of the article, I will briefly review other biochemical and biophysical data, which continues to accumulate rapidly, allowing interesting comparisons with structural data and helping to fill the gap between systems where structural information is available and systems where such information does not exist yet.

Adenylate and related complexes: structural results

Class II aaRSs

The recent completion of the refinement of the ^{sc}AspRS–tRNA^{Asp} complex in the presence of ATP [14••] allowed the visualization of a bent conformation for ATP, showing the leaving pyrophosphate group pointing away from the α-phosphate–ribose line and stabilized by at least one Mg^{2+} ion. Several crucial residues were identified, all of which are strictly conserved in class II aaRSs. Two acidic residues were found to bind a Mg^{2+} involved in the binding of the β- and γ-phosphates of ATP. The counterparts of these residues are found in all class II aaRSs (Fig. 1).

Soaking the ^{sc}AspRS–tRNA^{Asp} complex with ATP and aspartic acid leads directly to formation of the

Abbreviation
aaRS—aminoacyl-tRNA synthetase.

Table 1. Classification of aminoacyl-tRNA synthetases.

Class II	Quaternary structure	References	Class I	Quaternary structure	References
Gly	($\alpha_2\beta_2$)	[26•]			
Ala	(α_4)				
Pro	(α_2)				
Ser	(α_2)	[6,11•,12••,13••]			
Thr	(α_2)				
His	(α_2)	[27•]			
Asp	(α_2)	[7,14••,15•,16••]	Glu	(α)	
Asn	(α_2)		Gln	(α)	[4,9••]
Lys	(α_2)	[28]	Arg	(α)	
			Cys	(α_2)	
			Met	(α_2)	[3]
			Val	(α)	
			Ile	(α)	
			Leu	(α)	
Phe	($\alpha_2\beta_2$)	[17••]	Tyr	(α_2)	[1,2]
			Trp	(α_2)	[10••]

Classification of all aaRSs on the basis of the presence of the two signature sequences (HIGH and KMSKS; one letter amino acid code used) for class I and the sequence motifs 1, 2 and 3 for class II. On the structural level this corresponds to the presence of a Rossmann fold parallel β-sheet for class I and an antiparallel β-sheet for class II [8]. On the functional level, aminoacylation invariably occurs on the 2'OH of adenosine 76 of tRNA for class I aaRSs, and on the 3'OH for class II aaRSs (except for PheRS, which is aminoacylated on the 2'OH; in CysRS and TyrRS aminoacylation might occur on either of the OH groups). The quaternary structure (in *E. coli*) of all these enzymes is also shown; it is generally well conserved across species ($^{\alpha}$GlyRS, however, is an α_2 dimer). Subgroups corresponding to both sequence alignments inside each class and physico-chemical properties of the amino acid have been drawn.

aminoacylated tRNA in the crystal. The amino acid binding site could be identified, with a positively charged residue (Arg483) located at its base making an ion pair with the acidic group of the substrate side chain. A strictly conserved negatively charged residue was found to be responsible for maintaining the amino group of the amino acid in place (Fig. 1).

To observe experimentally the aspartyl–adenylate intermediate (first step of the reaction), it is necessary to soak crystals of the enzyme alone; this was done with $^{\alpha}$AspRS [16••], for which data for the structure of the enzyme alone also exist [15•]. It appears that the amino acid binding pocket is essentially rigid, with the essential Arg483 held in place through a network of electrostatic interactions with at least three other charged side chains. Redesign of the amino acid binding pocket of AspRS to make it accept lysine instead of aspartate would of course involve the mutation of Arg483 into a negatively charged amino acid (as is the case for LysRS, as shown by sequence alignment). But this is not enough, as this negative charge would then be in a very unfavourable environment; in fact, the whole pocket has to be electrostatically redesigned. This discussion is very close in spirit to the arguments developed more quantitatively by Hwang and Warshel [18], in the more general context of protein engineering. It will be interesting to see in the structure of LysRS how nature solved this problem [19•].

The structures of SerRS in complex with two analogues of seryl-adenylate [12••] show a marked similarity to AspRS in the active site: if one superimposes the α-carbon coordinates of the two enzymes and then applies this transformation to bring the aspartyl-adenylate into the framework of SerRS, the α-phosphate lies at exactly the same place as the one seen in the two analogues of seryl-adenylate (Fig. 2). The adenine ring is slightly shifted, but all the interactions described above for AspRS (except those concerning side-chain recognition) still hold for SerRS: in fact, the residues that are class II invariant lie at the same position, even though the backbone and the rest of the structure can vary (Fig. 2). There is some discrepancy concerning the location of the magnesium-binding site, but this could be explained by the fact that different stages of the reaction are observed and/or by the presence of more than one Mg^{2+} ion.

The first step in the charging of a tRNA is the formation of an aminoacyl–adenylate; a simple mechanism of in-line attack on the α–β phosphodiester bond of the ATP by the carboxyl group of the amino acid has been proposed [14••]. The transfer of this activated aminoacyl–adenylate species to the tRNA probably proceeds through an attack by the 3'OH of the ribose moiety of adenosine 76 of the tRNA, from which the proton has been extracted by one of the free oxygens of the α-phosphate of the adenylate. Such an attack, which does not involve any proton attractor from the protein itself, is not uncommon in enzymatic mechanisms: indeed, a similar mechanism has already been postulated for EcoRV, aspartate carbamoyl transferase and, more recently, the ^{ras}21 protein [20].

The tRNA-binding mode of SerRS has also been elucidated in the past year [13••], providing detailed information on the structure of the tRNA itself (which contains an unusual extra loop) and its interactions with the two very long and flexible helices at the N-terminal end of the protein. Unfortunately, the acceptor end of the tRNA appears to be disordered in the crystal of the complex, but its interaction with the synthetase is expected to be qualitatively similar (but different in detail) to the one observed in the AspRS system [21••], as revealed by a structural alignment between the α-carbon coordinates of both SerRS and AspRS.

It will be interesting to see if the mechanism postulated in [14••] can also be applied to PheRS, which aminoacylates the 2'OH of tRNA (an exceptional feature for

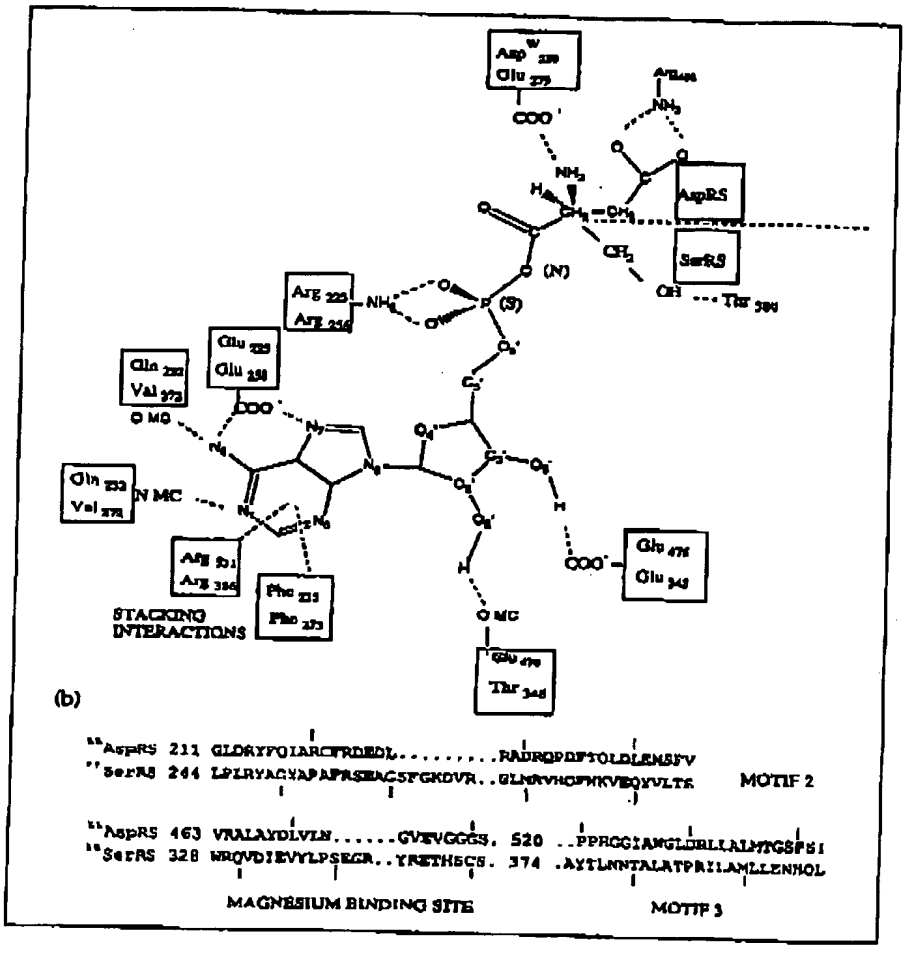

Fig. 1. Binding of aminoacyl-adenylate by class II aaRSs. (a) The interactions involved in the binding of the aspartyl-adenylate in ^{a}AspRS [16••], superimposed on the interactions involved in the binding of the inhibiting analog of the seryl-adenylate in ^{a}SerRS [12••]. The residues involved are boxed, with the residue from ^{a}AspRS shown uppermost and that from ^{a}SerRs shown below. The nature of their interaction with the aminoacyl-adenylate is shown: O(N) MC indicates hydrogen-bonding to the main chain oxygen (nitrogen); W (superscript) indicates that interaction is mediated by a water molecule. If implicated, the side chain of the amino acid is represented. The numbering in the two different proteins is also indicated (subscript) and corresponds to the alignment given below. (b) Alignment of AspRS and SerRS sequences. For simplicity, this alignment is restricted to the region of motifs 2 and 3 and to a newly identified conserved region, which, at least in AspRS, is implicated in Mg^{2+} binding; the issue of the presence of different ion binding site(s) in SerRS still has to be resolved. The residues indicated (in bold) have their side chains in the same spatial position in the two enzymes and are directly superimposable, even though some significant deviations occur in the rest of the backbone of the active site (e.g. region 384–386 in ^{a}SerRS, probably due to the presence of a proline).

a class II aaRS); its three-dimensional structure determination is near completion [17••].

Class I aaRSs

The recently solved structure of TrpRS in complex with tryptophanyl-adenylate [10••] reveals a pattern of interactions that closely matches that of TyrRS with its different substrates (tyrosyl-adenylate or tyrosine [1,2]). There is an almost perfect 1:1 functional correspondence between structurally equivalent residues in the two systems, even though the overall sequence identity between the two enzymes is very low (13% for 272 amino acids). The adenylate and amino acid binding pockets are clearly superimposable between the two structures. The tryptophan-binding pocket shows good steric complementarity with the substrate side chain, suggesting that it should be amenable to specificity modification using the normal principles of protein structure formation, in this case, essentially by alteration of side-chain packing. Redesigning the amino acid specificity of TyrRS is, however, not a trivial matter [22].

The structure of the TrpRS enzyme also contains information that could lead to a plausible model of its interaction with its cognate tRNA that would be consistent with the one previously proposed for TyrRS [23], with the acceptor and anticodon arms of the same tRNA interacting with two different monomers. The anticodon end of the tRNA would be undistorted in both TyrRS and TrpRS, contrary to what is observed in the GlnRS complex [4]. It should be recalled, however, that the tRNA-binding mode of MetRS has been linked to the one observed with the GlnRS system, especially because of the presence of a so-called acceptor binding domain [24], which is absent in both TyrRS and TrpRS. Also, there is a sixth strand with a left-handed type of connection in both MetRS and GlnRS that is absent both in TyrRS and TrpRS, and this might be related to a differ-

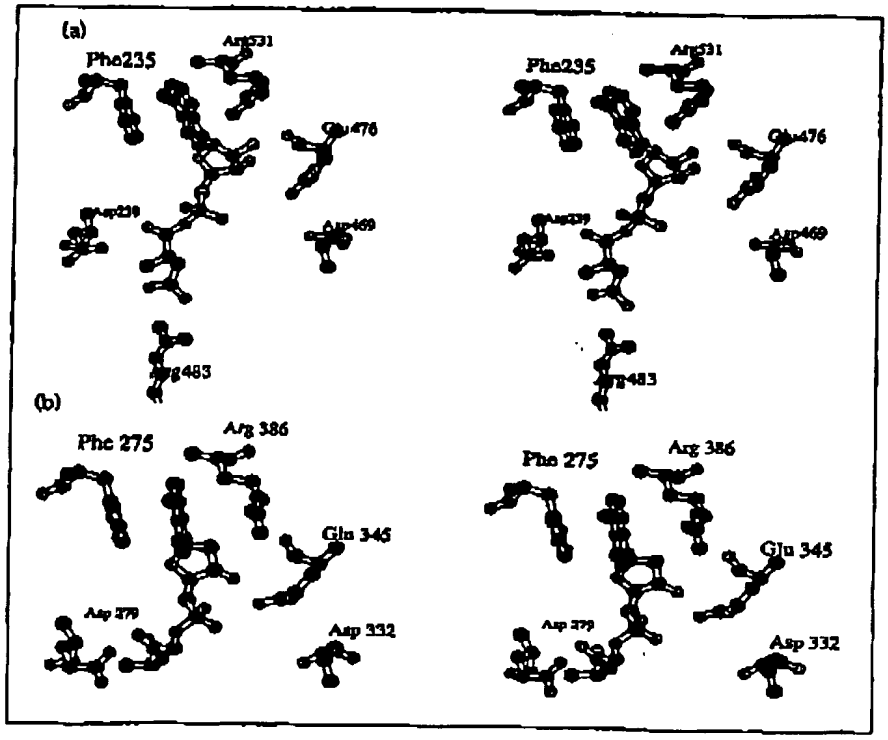

Fig. 2. MolScript stereo representation of the aminoacyl adenylate binding sites of (a) AspRS and (b) SerRS, as described in [12••] and [16••], respectively. For clarity, Arg223 and Arg256 (ligands of the α-phosphate in AspRS and SerRS, respectively) have been omitted. The two structures have been superimposed by using the α-carbon coordinates only, transporting the adenylate with the derived transformation afterwards.

ent tRNA-binding mode (both GlnRS and MetRS can function as monomers, whereas TyrRS and TrpRS are both functional dimers).

Last year, Perona *et al.* [9••] used crystallographic data on the complex of GlnRS–$tRNA^{Gln}$ bound to Mn^{2+} and ATP to infer the location of the amino acid binding pocket and to build a detailed enzymatic mechanism for the two-step reaction catalyzed by class I aaRSs. The striking conclusion of their work is that the second step is done in very much the same way as by the class II aaRSs, only in a different geometry: the extraction of a proton from the 2'OH of adenosine 76 of tRNA is also done by a free oxygen of the adenylate α-phosphate.

Perona *et al.* [9••] make a number of valuable comparisons between structural and functional results available for GlnRS, TyrRS or MetRS. A number of points that appear to be shared with class II aaRSs could also be highlighted; however, a word of caution is needed here. Even though the chemical nature of the amino acid side chains involved in the different interactions in both class I and class II aaRSs often appears to be comparable (see below), their positions are not strictly superimposable: indeed, if one superimposes the adenine rings, the aminoacylation reaction appears to take place on opposite sides [14••].

The first common point is that the way the adenine ring of ATP is recognized in GlnRS is highly reminiscent of what is seen in both SerRS and AspRS: the N1 and N6 atoms are bound to the amide nitrogen and the carbonyl oxygen of the main chain of the same residue, namely Leu261 in GlnRS (compare with Fig. 1). Also, the adenine ring rests upon the aliphatic portion of Arg260, in very much the same way as in class II aaRSs (Arg531 of ^{ec}AspRS). Actually, Perona *et al.* [9••] point out that this might be functionally important: as the side chain of arginine is highly flexible, this might allow without difficulty a slight rearrangement of the adenine ring moiety during the different steps of the reaction.

Another interesting feature is that the adenine ring lies on Gly42, a strictly conserved residue of the first signature sequence of class I aaRSs; any other amino acid side chain would interfere with the binding of ATP. Other strictly conserved residues of either the first (His43) or the second (Lys270) signature sequence of class I aaRSs are implicated in the stabilization of the transition state of the reaction, which involves a pentavalently bonded phosphorus. This is to be related with earlier work using kinetic measurements by Fersht *et al.* (reviewed in [9••]). Lys270 can be compared to Arg223 of motif 2 in ^{ec}AspRS in its role in binding the α-phosphate.

The α-amino group of the amino acid appears to be stabilized by an ion pair with an aspartate, which is conserved in all (except TyrRS) class I aaRSs, at the end of

β-strand C; again, this is comparable with what is seen for class II aaRSs.

The binding pocket for glutamine in GlnRS contains Arg30, Tyr211 and Gln255 at its base, the glutamine being ready to hydrogen bond to the substrate. Gln255 is held in place by a network of interactions within a polar environment involving the charged side chain of Glu257 and the main chain carbonyl atom of Phe233. This situation is highly reminiscent of the binding pockets of both AspRS [16••] and TyrRS [2,22]. All of this has been observed in the structure of GlnRS free of glutamine, thereby implying that the free enzyme can bind its substrate without any significant rearrangement (no induced fit). It is to be hoped that the general nature of all the observations mentioned above will be confirmed by the detailed structural studies on αGluRS and αMetRS (S Yokoyama, personal communication).

AaRS–tRNA recognition: modules and nodules

One of the most useful concepts in analyzing aaRSs of unknown structure remains the concept of modularity, originally introduced for *Escherichia coli* AlaRS [25]. Apart from their catalytic domain, aaRSs often possess additional domains that help to make them more specific in the tRNA recognition process. Very often, these domains can be identified through sequence analysis [26•]. New sequences of aaRS genes in different organisms continue to appear, but are too numerous to be cited in this review.

Structural data on the different domains found in aaRSs have also appeared, and it is not inconceivable that we will soon have a complete picture of the repertoire used by this family of enzymes. The structure of αAspRS [15•] revealed an additional domain, of unknown function, structurally similar to the histidine-containing phosphocarrier protein HPr. The structure of αGluRS may reveal a different anticodon-binding domain, compared with GlnRS. The structures of αGlyRS [27•] and αβHisRS [28•], now approaching complete refinement (D Moras, personal communication), will also provide the first picture of a new anticodon-binding domain that should also be present in ThrRS. It will be interesting to compare the forthcoming structure of αβLysRS [19•] with AspRS, especially their amino acid binding pockets. In addition, the role of the N-terminal extension of mammalian aaRSs is being actively studied [29].

How these domains interact with each other is still a daunting question. This question relates, of course, to the enzymes' specificity and, in some cases, allostery. Some progress has been made in the class II aaRS family through a careful structural analysis of the dimer interface [15•,30•] and through probing by site-directed mutagenesis [30•]. In the class I family, some intragenic suppressors that compensate for impaired aminoacylation in mutants of GlnRS have been isolated and structurally mapped to a region linking the catalytic and the anticodon-binding domains [31•].

The structure of the zinc-finger domain of MetRS has also been solved recently by NMR methods [32•]. This is especially important because zinc-finger domains have been identified in many different class I aaRSs by sequence analysis.

Meanwhile, experiments designed to probe tRNA–aaRS interactions and to define the tRNA identity [33•] are producing interesting new results. In some cases, it proved possible to change the specificity of the recognition [34]. The structure of the tRNA itself and its role in aaRS recognition continues to be actively studied [35–37]. The role of post-transcriptional modifications of tRNA in the recognition process has also been recently re-emphasized [38]. The part of the protein responsible for recognition can in some cases be pinned down to a few residues [39] and the switch to another specificity can involve as little as one methyl group [38], hence the notion of a nodule.

AaRSs may represent a unique system, as far as protein engineering is concerned, because the structure of three different members of this family are (or are about to be) known in both *E. coli* and *T. thermophilus*, namely MetRS, SerRS and AspRS; the *E. coli* AspRS has also recently been solved (D Moras, personal communication). Thus, the study of aaRSs may represent one of the best opportunities to understand the molecular basis for thermophilicity. An interesting experimental approach has been undertaken with TyrRS [40], whose structure is known in the thermophilic organism *B. stearothermophilus*. Chimeric versions of TyrRS were constructed using portions of sequence from the *E. coli* and *B. stearothermophilus* enzymes, and the thermal stability of the resulting enzymes measured.

Other biophysical and biochemical studies

Although the amount of information given by these structural results is impressive, more data are still needed to refine our understanding of how enzymes of this family function. As usual, sequence data complement refined structures well because sequence conservation profiles help to identify functionally important residues [26•]. Information from the multiple alignment of AlaRS sequences, secondary structure predictions and site-directed mutagenesis experiments has been used to identify the position of motif 2 in AlaRS [41,42].

To check hypotheses about the reaction mechanism suggested by crystal structures (which only give snapshots of some intermediate states of the reaction), it is often instructive to generate single point mutants and measure their functional and kinetic properties [43–45,46•]. Chemical affinity labeling followed by microsequencing of the labelled peptides [47] has also proved useful in identifying residues in contact with the active site, as have

other biochemical and biophysical measurements such as fluorescence studies [48].

One unresolved issue that is receiving more attention is the mechanism of proofreading. It has been known for a long time that in some instances misactivation can occur; however, upon tRNA binding, hydrolysis of the misactivated amino acid takes place, so preventing the tRNA from being charged with the wrong amino acid. Site-directed mutagenesis in the inferred amino acid binding pocket of MetRS [49] has been used to study this issue, but a structural picture of the 'hydrolysis pathway' for a misactivated amino acid remains elusive. For ValRS [50•], a mutant has been engineered with decreased ability to distinguish between the right (valine) and the wrong (isoleucine) amino acid. In any case, systematic measurements of all the kinetic and binding properties of an aaRS amino-acylating (and charging) all possible amino acids are certainly welcome [51,52•] and should be made in the systems where detailed structural information is already available. This work in turn should prove useful in calibrating molecular dynamics simulation methods applied to these systems [53].

Conclusion

The past year has seen the elucidation of the structures of several aaRSs (from both class II and class I) with their different substrates. Striking and unanticipated structural and functional similarities have been revealed, opening the way for the rational design of mutants and of aaRSs with changed specificity. This will probably demand more sophisticated molecular modelling methods than the already existing ones. In addition, structural data on the same enzyme from both bacteria and eukaryotes (as is available for AspRS) may prove useful for the design of inhibitors specific for prokaryotes. In this respect, an interesting experiment in bacteria has recently been done [54•]. Expression of an intentionally inactivated tRNA synthetase, proved effective (and lethal) upon introduction of the gene encoding it into the genome of *E. coli*; this type of effect had actually already been observed for TyrRS [55].

Data on a peculiar (and puzzling) feature of tRNA-synthetases, namely the antigenic nature that they sometimes show, continue to accumulate. ValRS, a class I aaRS, has recently been identified as one of the major antigenic determinants of *Plasmodium vivax* (V Snewin, personal communication). IleRS has also been implicated in several autoimmune diseases, for instance in the rheumatic disease myositis [56]. Antibodies against class II aaRSs have been found as well (e.g. HisRS, GlyRS, AlaRS and ThrRS in myositis and AsnRS in filariosis [8]). Therefore, our knowledge of aminoacyl–tRNA synthetases may eventually have medical applications.

Note added in proof

It has recently been found that the class II aaRS catalytic domain fold is present in BirA, a biotin-protein ligase that attaches biotin to various key metabolic proteins involved in carboxylation and decarboxylation [57•]. The biotination reaction proceeds through the same type of intermediate as found in aaRSs, namely an acyl-adenylate. It is to be expected that the same type of fold will be observed in yet other proteins involved in metabolism, for instance acetyl CoA ligases.

Acknowledgements

I wish to thank P Akzari, H Bedouelle, D Logan and D Moras for useful comments on the manuscript. Special thanks to CW Carter for communicating a copy of his manuscript prior to publication.

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Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

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9. •• Perona JJ, Rould MA, Steitz TA: Structural basis for tRNA aminoacylation by *E. coli* glutaminyl-tRNA synthetase. *Biochemistry* 1993, 32:8758–8771.
This mammoth paper is likely to be the reference on detailed mechanistic and structural studies of class I aaRSs for quite some time. Apart from

new structural results on the GlnRS–tRNA^{Gln} in complex with various substrates, it contains a brilliant synthesis of all previously known structures and kinetic measurements of class I aaRSs and related complexes. It can now be updated with [10••]; many of the ideas developed here may find a counterpart in class II aaRSs (see [14••]).

10. •• Doublié S, Bricogne G, Gilmore C, Carter CW Jr: **Tryptophanyl-tRNA synthetase crystal structure reveals an unexpected homology to tyrosyl-tRNA synthetase.** *Structure* 1995, 3:17–32.

Description of the structure of another class I aaRS, TrpRS, as a complex with tryptophanyl-adenylate. The paper also describes in detail how this complex compares with the TyrRS results in [2]. In addition, it contains an interesting discussion of a new approach in crystallographic structure resolution, based on a maximum likelihood criterion, to find the phases of strong reflections and correct the envelope in the initial stage of model building.

11. • Fujinaga M, Berthet-Colominas C, Yaremchuk AD, Tukalo M, Cusack S: **Refined crystal structure of seryl–tRNA synthetase from *Th. thermophilus* at 2.5 Å resolution.** *J Mol Biol* 1993, 234:257–280.

This paper contains an interesting discussion concerning the possible molecular basis of the thermophilicity of this enzyme. It also emphasizes the flexibility of the two long α-helices in the N-terminal part of the sequence.

12. •• Belrhali H, Yaremchuk A, Tukalo M, Larsen K, Berthet-Colominas C, Leberman R, Beijer B, Als-Nielsen J, Grubel G, Legrand JF *et al.*: **Crystal structure at 2.5 Å resolution of seryl-tRNA synthetase complexed with two analogs of seryl adenylate.** *Science* 1994, 263:1432–1436.

The fact that the two non-hydrolyzable analogs bind at the same place in the enzyme provides strong support for the hypothesis that they actually reflect the binding of native seryl-adenylate. The network of interactions between the protein and the substrates is carefully analyzed and described. The authors also cite recent data at 2.4 Å resolution on a complex between the protein and ATP and Mn^{2+}, which shows the ATP in a bent conformation, as observed in [14••].

13. •• Biou V, Yaremchuk A, Tukalo M, Cusack S: **The 2.9 Å crystal structure of *Thermus thermophilus* seryl–tRNA synthetase complexed with tRNA^{Ser}.** *Science* 1994, 263:1404–1410.

This paper gives structural details of the interactions between SerRS and its cognate tRNA, showing that it is the special feature of the tRNA, namely the extra long loop, and not the anticodon, that is recognized by the protein. Unfortunately, the CCA end appears to be disordered in the crystal. Data have also been collected in the presence of the ATP analog AMPPCP, which, contrary to the ATP, is in an extended conformation; this is in accordance with results also described in [14••].

14. •• Cavarelli J, Eriani G, Rees B, Ruff M, Boeglin M, Mitschler A, Martin F, Gangloff J, Thierry JC, Moras D: **The active site of yeast aspartyl-tRNA synthetase: structural and functional aspects of the aminoacylation reaction.** *EMBO J* 1993, 13:327–337.

Class II counterpart of [9••]. It describes for the first time the binding of ATP to a class II aaRS, as well as a complex of a class II aaRS with an aminoacylated cognate tRNA; all class II invariant residues implicated in the active site are identified and a mechanism is proposed. Several mutants have been constructed and their functional properties measured to check this mechanism. These results are to be supplemented by the experimental determination of aspartyl-adenylate bound to ^{Sc}AspRS described in [16••]. See also [12••,13••].

15. • Delarue M, Poterszman A, Nikonov S, Garber M, Moras D, Thierry JC: **Crystal structure of a prokaryotic aspartyl tRNA-synthetase.** *EMBO J* 1994, 14:3219–3229.

Description of the structure of ^{Tt}AspRS; an additional domain is present, as compared to the yeast enzyme, which presents the same topology as protein HPr (of the *E. coli* phosphotransferase sugar transport system) thereby making this structure a textbook example of the modularity of aaRSs. A detailed description of the dimer interface is also included, as well as a proposal of a structural communication pathway between the catalytic and the anticodon-binding domains. See also [16••].

16. •• Poterszman A, Delarue M, Thierry JC, Moras D: **Synthesis and recognition of aspartyl-adenylate by *Thermus thermophilus* aspartyl-tRNA synthetase.** *J Mol Biol* 1994, 244:158–167.

First structure of a class II aaRS with the cognate (native) adenylate, which in a sense represents the situation after the first step of the reaction has been completed. A comparison with the structure of the enzyme alone (see [15•]) is also given, showing the rigid nature of the amino acid binding pocket.

17. •• Mosyak L, Safro M: **Phenylalanyl–tRNA synthetase from *T. thermophilus* has four antiparallel folds of which only two are catalytically active.** *Biochimie* 1993, 75:1091–1098.

This paper describes the preliminary chain tracing of ^{Tt}PheRS; the unexpected result is that the β-subunit (the largest one) also contains the typical fold of class II aaRSs, i.e. an antiparallel β-sheet, even though sequence analysis could not locate the three motifs characteristic of this class. It turns out that this subunit is probably not functional, but has evolved from the same ancestor as that of all class II aaRSs. The authors also propose a convincing location in the α-subunit for motif 1, which had been missed by earlier studies. The α-subunit also contains the fold typical of class II aaRSs. The dimerization mode between the α- and β-subunit is similar to the α_2 dimerization interface observed in both AspRS and SerRS.

18. Hwang JK, Warshel A: **Why ion pair reversal by protein engineering is unlikely to succeed.** *Nature* 1988, 334:270–272.

19. • Onesti S, Theocilitou ME, Wilzung PL, Miller AD, Plateau P, Blanquet S, Brick P: **Crystallization and preliminary diffraction studies of *E. coli* lysyl–tRNA synthetase.** *J Mol Biol* 1994, 243:123–125.

Crystals diffracting to 2.1 Å resolution have been obtained for *E. coli LysU* gene product. The structure of the protein is to appear soon.

20. Schweins T, Langen R, Warshel A: **Why have mutagenesis studies not located the general base of ras p21?** *Nature Struct Biol* 1994, 1:476–484.

21. •• Cavarelli J, Rees B, Ruff M, Thierry JC, Moras D: **Yeast tRNA^{Asp} recognition by its cognate class II aminoacyl-tRNA synthetase.** *Nature* 1993, 362:181–184.

The refined structure at 2.9 Å resolution of this tRNA–aaRS complex allows a more detailed description of the interactions between the tRNA and the protein. The results are analyzed in light of other biochemical experiments. The generalization of the results to other class II aaRSs is discussed (see also [13••]).

22. De Pras Gay G, Duckworth HW, Fersht AR: **Modification of the amino acid specificity of tyrosyl–tRNA synthetase by protein engineering.** *FEBS Lett* 1993, 318:167–171.

23. Bedouelle H, Guez-Ivanier V, Nagaotte R: **Discrimination between tRNAs by tyrosyl-tRNA synthetase.** *Biochimie* 1993, 75:1099–1108.

24. Perona JJ, Rould MA, Steitz TA, Risler JL, Zelwer C, Brunie S: **Structural similarities in glutaminyl- and methionyl-tRNA synthetases suggests a common overall orientation of tRNA binding.** *Proc Natl Acad Sci USA* 1991, 88:2903–2907.

25. Jasin M, Regan L, Schimmel P: **Modular arrangement of functional domains along the sequence of alanyl-tRNA synthetase.** *Nature* 1983, 306:441–447.

26. • Delarue M, Moras D: **The aminoacyl-tRNA family: modules at work.** *BioEssays* 1993, 15:675–687.

A recent review on aaRSs, with special emphasis on the modularity and interplay between structural results and multiple sequence alignments.

27. • Logan DT, Cura V, Touzel JP, Kern D, Moras D: **Crystallization of the glycyl-tRNA synthetase from *T. thermophilus* and initial crystallographic data.** *J Mol Biol* 1994, 241:732–735.

Native and derivative data on two crystal forms have been obtained. An interpretable map has been produced.

28. • Francklyn C, Harris D, Moras D: **Crystallization of histidyl-tRNA synthetase from *E. coli*.** *J Mol Biol* 1994, 241:275–277.

Crystals in two forms have been obtained in the presence of histidine and ATP. The refined structure should be available soon.

29. Kovaleva G, Nikitushnika T, Kisselev L: **Nucleoside triphosphatase activity associated with the N-terminal domain of tryptophanyl-tRNA synthetase.** *FEBS Lett* 1993, 335:198–202.

30. • Eriani G, Cavarelli J, Martin F, Dirheimer G, Moras D, Gangloff J: **Role of dimerization in yeast aspartyl-tRNA synthetase and importance of the class II invariant proline.** *Proc Natl Acad Sci USA* 1993, 90:10816–10820.

The role of the class II invariant Pro273 in motif 1 was investigated by site-directed mutagenesis and analyzed in light of the structure of the ^{Sc}AspRS–tRNA^{Asp} complex.

31. Rogers MJ, Adachi T, Inokuchi H, Soll D: **Functional communication in the recognition of tRNA by *E. coli* glutaminyl-tRNA synthetase.** *Proc Natl Acad Sci USA* 1994, 91:291–295.
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There is some discrepancy between this NMR-derived structure of the zinc-binding domain of *E. coli* methionyl-tRNA synthetase and the published crystal structure [3]. The crystal structure of the *Th. thermophilus* enzyme should soon resolve this issue.

33. McClain WHM: **Rules that govern tRNA identity in protein synthesis.** *J Mol Biol* 1993, 234:257–280.
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41. Davis MW, Buechter DD, Schimmel P: **Functional dissection of a predicted class-defining motif in a Class II tRNA synthetase of unknown structure.** *Biochemistry* 1994, 33:9904–9911.

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This article shows that birA, a biotin-protein ligase, and class II amino acid tRNA synthetases are structurally homologous in their catalytic domains.

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